

## Wnt Signaling Related to Subchondral Bone Density and Cartilage Degradation in OA

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The role of subchondral bone in the progression of osteoarthritis has been controversial for nearly 50 years.<sup>(1,2)</sup> The observation that subchondral sclerosis was nearly always present in end-stage disease led to the conclusion that the increased stiffness caused by a thicker subchondral bone plate detracted from the bone's ability to attenuate the loads imposed on the joint cartilage, increasing cartilage stresses and initiating the process of joint deterioration. Because cartilage damage does not always progress to full thickness cartilage loss and OA, Radin and Rose<sup>(3)</sup> proposed that the initiation and the progression of cartilage deterioration were separate processes. They suggested that it was steep stiffness gradients at the joint margins, primarily regions of high tensile and shear stresses that create inhomogeneities in the subchondral plate/cartilage complex. They hypothesized that these changes caused differential deformation between more compliant and denser regions of the subchondral bone, tearing the cartilage attached to it and initiating fibrillation. They further proposed that progression of cartilage loss, a separate process, ensued when continued

loading occurred on an already dense and stiff subchondral plate. Several animal models support this progression of events. In a model of spontaneous age-related OA in cynomolgus macaques much like that which occurs in people, Carlson et al.<sup>(4,5)</sup> clearly demonstrated that cartilage damage occurs subsequent to subchondral bone densification.

The paper by Jia et al.<sup>(6)</sup> examines the mechanisms for subchondral densification, and whether increased subchondral plate density is a *sine qua non* of progressive disease. To do this, they employed several different mouse models, and induced OA with two different surgical approaches. They first developed a cartilage-specific knockout of the epidermal growth factor receptor (Egfr CKO), which they had previously shown regulates chondrocyte number and joint lubrication.<sup>(7,8)</sup> They surgically destabilized the medial meniscus (DMM), and in some cases hemi-sected the meniscus (DMMH) in Egfr CKO or WT mice, observing changes after 2-3 months. To simulate the effects of aging, the authors performed DMM on WT mice, observing cartilage changes 10 months after surgery. They also examined the spontaneous initiation and progression of OA in Egfr CKO mice over a 12 month period. To determine the effect of pre-existing bone densification on the process of cartilage degeneration, they also subjected SOST KO mice to DMMH. This directly tests the Radin and Rose hypothesis that increased density and stiffness of the subchondral bone are a necessary pre-condition for progression of OA. If they are, then one might expect an accelerated onset of OA, or cartilage degeneration that is more severe than in WT mice. If they are not a pre-condition, then the expectation is that cartilage degeneration and the development of OA will occur similarly in those mice without pre-existing subchondral densification, or not occur at all. The authors utilized a novel microCT approach that allowed them to visualize bone and cartilage changes three-dimensionally, rather than by 2D histological serial sectioning. Given the focal nature of cartilage degeneration in OA, 2D histological approaches can provide an imperfect view of the relationship between cartilage and bone in the regions where cartilage fibrillation occurs. This new method allowed the authors to localize subchondral bone changes to those areas in which cartilage was either healthy or degenerating.

What they observed in those models undergoing DMM surgery was a location-specific increase in subchondral plate thickness, occurring from the marrow side of the subchondral plate, but only at sites of cartilage deterioration and not at sites where the overlying cartilage was healthy. This was associated with a localized reduction in sclerostin in subchondral bone, increased vessel ingrowth, and more osteoblasts, but only at those sites associated with increased thickness and cartilage damage. These areas were in load-bearing sites of the joints; non-loading bearing sites did not show similar cartilage or bone changes. This

implicates mechanical loading as complicit in the degenerative process. In situations in which there was not cartilage damage, and in non-loadbearing sites, these subchondral plate changes did not occur. Interestingly, spontaneous age-related development of cartilage damage in the Egfr CKO mice was not localized to the medial weight bearing sites, but was also found laterally in association with reduced sclerostin. As has been observed in other models in which subchondral plate sclerosis occurs in association with cartilage loss,<sup>(9)</sup> the trabecular bone adjacent to the subchondral plate becomes osteopenic and its microarchitecture deteriorates, probably due to the effects of stress shielding.

The authors conclude that subchondral bone sclerosis occurs through a downregulation of sclerostin that is fueled by mechanical loading in those regions of the joint in which cartilage is breaking down. This is consistent with what we know about the role of sclerostin in mechanically-induced bone formation from animal models subjected to in vivo mechanical loading.<sup>(10)</sup> Two months after DMM surgery, changes in proteoglycan and cartilage fibrillation can be seen, at a time when the authors were unable to identify any changes in the subchondral bone. Based on this, they conclude that subchondral sclerosis is secondary to the cartilage change, and is driven by the mechanically-mediated down-regulation of sclerostin. As further evidence of this, they note that SOST KO mice do not develop spontaneous cartilage deterioration, even in mature 14-month-old mice, despite the fact that downregulation of SOST results in a significant increase in bone volume and density.<sup>(11)</sup> This is consistent with observations in people who have a gain of function mutation of the lipo-protein receptor-related protein 5 (LRP5) gene that leads to high bone mass.<sup>(12)</sup> However, there is no evidence of increased incidence of OA in individuals with this mutation compared to the normal population, showing that sclerotic subchondral bone alone does not lead to cartilage disease. Animal models also have shown that the existence of elevated subchondral density alone does not lead to cartilage deterioration and OA, unless preceded by a period of increased subchondral remodeling and vascularization. The Dunkin-Hartley guinea pig model exhibits a high rate of subchondral bone turnover within the first two months of life, and develops severe OA progressively over 12 months.<sup>(13)</sup> A second guinea pig strain, Strain 13, which, at 2 months of age has a subchondral plate thickness 6-7 times that of the Dunkin-Hartley model, does not have a high rate of subchondral bone turnover, and does not develop evidence of OA. All these lines of evidence show that subchondral plate densification by itself does not initiate joint disease, and the authors make this clear when they state: "SBP thickening alone is not sufficient to affect cartilage degeneration, and . . . cannot be an initiator of cartilage and joint damage in OA."

When DMMH is performed on SOST KO mice, the resulting cartilage deterioration is no worse than it is in WT mice, suggesting that subchondral sclerosis is not driving the progression of joint degeneration either, but rather reacting to it. That pre-existing subchondral sclerosis does not accelerate the process of joint degeneration or make the OA worse may detract from the thesis of Radin and Rose that subchondral sclerosis is required for progression of disease once cartilage deterioration has initiated. But it is not conclusive as subchondral sclerosis either exists (SOST KO) or develops (WT) during the process of joint destruction. Evidence that subchondral sclerosis does not contribute to joint deterioration would only be conclusive if it could be shown that OA develops even in the absence of subchondral sclerosis. Therefore, an experiment like the ones in the Jia study in which subchondral bone is prevented from becoming sclerotic (perhaps by performing DMMH in a SOST overexpressing mouse, or by preventing increased subchondral plate thickness), would provide the negative control needed to conclusively reject the hypothesis that subchondral sclerosis is a necessary component of progressive disease.

Sclerostin is also expressed by chondrocytes,<sup>(14)</sup> however, and so changes in Wnt signaling may also be important to cartilage health either independent of, or concurrent with, changes that occur in subchondral bone. A comparison of the expression of Wnt genes in bone samples from hip fractures and hip/knee OA showed upregulation of seven genes including LRP5 in the OA samples.<sup>(15)</sup> This led these authors to conclude that Wnt pathway genes not only affect the subchondral bone but also regulate cartilage degradation in OA. This is supported by earlier observations that increased  $\beta$ -catenin signaling was associated with an OA phenotype in a conditional overexpressing mouse model.<sup>(16)</sup> Wnt signaling can be induced by pro-inflammatory cytokines<sup>(17,18)</sup> and cause cartilage matrix degradation by stimulating the expression of matrix metalloproteinases (MMPs) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs).<sup>(19)</sup> Shin et al.<sup>(20)</sup> compared the expression of Lrp5, type II collagen and MMPs in human and mouse osteoarthritic cartilage, and found a relation between increased Lrp5 expression and cartilage destruction in osteoarthritis. On the other hand, Lrp5 deficiency decreased Wnt-mediated cartilage destruction. Similar findings were reported in a study that investigated Wnt signaling and Lrp5 expression in human osteoarthritic chondrocytes.<sup>(21)</sup> Increased Lrp5 expression was consistent with cartilage destruction whereas decreased levels reduced the extent of cartilage damage.

Joint instability (DMM) in SOST KO mice leads to higher OA scores, pointing towards the significance of sclerostin in maintaining cartilage integrity during mechanical loading.<sup>(22)</sup> Similarly, selective inhibition of the Wnt pathway by Dkk1 decreases the severity of OA.<sup>(23)</sup> However, in both sheep and mouse surgically-induced OA models, SOST was increased in

cartilage, but only focally in regions of cartilage damage, while being focally decreased in subchondral bone associated with the cartilage damage.<sup>(14)</sup> The upregulation of SOST in these cases seems somewhat contradictory to the idea that increased Wnt signaling promotes cartilage degradation. However, this could be a secondary response to the increased inflammation associated with the initiation of cartilage degeneration,<sup>(17,18)</sup> and therefore temporal relationships in SOST regulation and Wnt signaling are important to study. Preventing expression of SOST and allowing Wnt signaling will increase subchondral bone density and may permit the initiation of cartilage degradation, but later stage inflammatory processes could stimulate the production of SOST, leading to full thickness cartilage loss.

The observation that increased plate thickness occurs subsequent to the initiation of cartilage deterioration does not address whether other changes to subchondral bone that occur prior to overt cartilage deterioration contribute to the disease. Although Jia et al.'s work argues against the hypothesis of Radin and Rose that increased subchondral density drives progression of disease, this leaves open the question of what initiates the process in the first place. There is now accumulating evidence that increased remodeling in the early phases of joint disease may be required for progression of disease. This was noted many years ago by Li and Aspden<sup>(24)</sup> who showed that bone in patients with OA was significantly less stiff for a given apparent density than normal or osteoporotic bone. This does not mean that the subchondral plate is less stiff at a structural level, and it may not be. However, the tissue modulus is lower because of the high turnover and mineralization lag period prior to full maturation and mineralization of the tissue. Day et al.<sup>(25)</sup> showed that subjects who were in the early phases of cartilage loss but not yet arthritic had lower subchondral bone tissue modulus, confirming the earlier work of Li and Aspden. The increase in remodeling rate in the early phases of OA has been verified using animal models.

When this early remodeling reaction is suppressed using bisphosphonates<sup>(26-29)</sup> or RANKL inhibitors<sup>(30,31)</sup> that prevent the early increase in remodeling but eventually lead to increased subchondral plate thickness, cartilage is protected from progressive deterioration. The protective effect may be both dose- and time- related. More potent BPs appear to have a greater effect,<sup>(29)</sup> as does treatment earlier in the degenerative process.<sup>(32-34)</sup> Although suppression of remodeling is not effective at preventing all cartilage damage, early suppression is associated with prevention of progressive joint disease. These studies clearly demonstrate that increased bone remodeling is a precursor to OA, and that suppression of remodeling in early phase disease can prevent OA even if it leads to subchondral densification.

The work of Jia et al. is instructive in showing that mechanically-induced downregulation of sclerostin provides a mechanism for focally-increased subchondral bone density. Lower sclerostin expression has been shown in bone biopsies from OA patients compared to healthy controls,<sup>(35)</sup> but may not reflect processes involving Wnt signaling in cartilage.<sup>(36,37)</sup> In the models used by Jia, it would be fascinating to understand the temporal expression of SOST in cartilage, and associated changes in Wnt signaling. The data suggest an early decrease in sclerostin expression that leads to increased bone density but may also be permissive for cartilage degradation, with a subsequent inflammation-related increase in cartilage SOST expression. This Wnt signaling link makes the relationship between cartilage loss and subchondral bone densification more complex. Evidence that cartilage loss occurs prior to sclerostin downregulation and subsequent densification of subchondral bone has not been conclusively shown, and awaits a more thorough temporal analysis of both cartilage and bone changes in the models employed here. The argument over whether osteoarthritis initiates in the bone or the cartilage has not been resolved fully, as evidenced by a scheduled debate on the topic at the 2018 meeting of the Orthopaedic Research Society. One difficulty in resolving this question is that OA is a condition that takes a long time to develop. Therefore, it is difficult to know in humans, if and when early joint changes will progress to OA. It is also difficult to determine in those with OA when the process began, or what the initiating factor might have been. In this regard, there is a movement towards studying post-traumatic OA (PTOA) in which the time of initiation can be identified precisely. As PTOA progresses in a way that is consistent with age-related OA (at least macro- and microscopically), this is an appropriate way to develop new therapies, even though we don't really know that PTOA and age-related OA are equivalent mechanistically.

It has been suggested that OA is a final common pathway for a multi-etiological set of joint diseases and conditions that all lead to the same joint degenerative changes that involve both elevated subchondral density and cartilage deterioration and eventual loss. It is possible that the final common mechanistic pathway is through Wnt signaling, which both accelerates cartilage deterioration and underlies increased subchondral density. This may be the long sought link between bone and cartilage change that Radin and his colleagues attempted to identify 50 years ago. Based on data in the paper by Jia et al., Radin et al. appear to have been correct about the role of mechanical loading. However, at that time it was not possible to identify the molecular link for these events, which only became apparent recently with the discovery of the role of the Wnt pathway, and its inhibitors such as SOST and DKK.



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